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A FUNDAMENTAL ERROR IN CURRENT ATTEMPTS TO APPLY PHYSICAL CHEMISTRY TO SERUM PATHOLOGY.*

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IN so far as attempts to isolate bacterial toxins, antitoxins, and other specific immunity substances have proved unsuccessful, any indirect method by which light may be thrown on the nature of these important substances is welcomed by the biological chemist. The method that seems to promise most is the method of physical chemistry. The application of physico-chemical laws, therefore, has deservedly received the attention of numerous serum pathologists during the last few years.

The law of physical chemistry, whose application has apparently met with the greatest success, is the law of mass-action. When one examines, however, the experimental data on which the application of this law is based, one is struck with the numerous hypotheses such attempts necessitate. One hypothesis especially is fundamental in all these attempts. This hypothesis not only rests on no experimental basis, but experimental verification of it is practically out of the quesiton, till toxins, antitoxins, and the like are isolated in a state of purity. It is the purpose of the present paper to point out this fundamental error in pathological reasoning, and to emphasize the vicious rôle it is playing in current biological investigation.

The law of mass-action is well established for simple chemical substances. It has been shown, for instance, that if a certain amount of substance, dissolved in a given volume of solvent, undergoes chemical change at a given rate, twice the amount of the same substance, dissolved in the same volume of solvent, will change twice as rapidly; three times the amount, three times as rapidly; and so on; the amount changing per unit time being proportional to the amount present in the solution.

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To illustrate, if in a solution containing 100 grams of cane sugar, 6 grams are hydolyzed per minute to form glucose and fructose, then, other conditions being identical, in a solution of like volume containing but 50 grams, only half of 6 grams, or 3 grams, will be hydolyzed per minute; and in a solution containing 200 grams, twice 6 grams, or 12 grams per minute.

This does not mean that in the latter case 12 grams will be decomposed during every minute, but only that 12 grams will decompose during the first minute. At the beginning of the second minute, there will be present in the solution but 188 grams of unchanged sugar. During that minute, therefore, there will be decomposed but $\frac{188}{200}$ of 12 grams, or 11.28 grams. During the third minute, $\frac{176.72}{200}$ of 12 grams, or 10.6 grams. And so on.

The law of mass-action, as applied to the decomposition of cane sugar, may be expressed mathematically as follows:

$$[\text{Glucose}] = K \times [\text{Cane sugar}],$$

where the symbol [Glucose] stands for the amount of glucose formed per minute, the symbol [Cane sugar] for the amount of unchanged cane sugar present at the beginning of the minute, and K a constant whose value is determined by such things as the amount and nature of the solvent, the temperature, acidity, alkalinity, and the like. In the above illustration, $K = 6$ per cent.

This formula illustrates the law of mass-action as applied to a chemical change involving but a single chemical substance. If two chemical substances enter into combination, the law is slightly more complex. Here, the rate of reaction is proportional to the amount of each substance present in the solution, and is therefore proportional to their product.

Thus, if 100 grams of methyl acetate and 10 grams of potassium hydrate are dissolved in a liter of water, they will slowly react to form potassium acetate and methyl alcohol. If, under certain conditions, three grams of potassium acetate are formed during the first unit of time, doubling the amount of either the methyl acetate or the potassium hydrate present, will double the amount of the potassium acetate so formed, and doubling the amount of both will increase the amount fourfold. This law may be expressed mathematically as follows:

$$[\text{Potassium acetate}] = K \times [\text{Methyl acetate}] \times [\text{Potassium hydrate}]\,,$$

where the symbol [Potassium acetate] stands for the amount of potassium acetate formed per minute, the symbols [Methyl acetate] and [Potassium hydrate] for the amounts of the reacting substances present in the solution at the beginning of that minute, and K a constant, whose value is determined by such things as the volume and nature of the solvent, the temperature, and the like. In the above illustration, $K = 0.3$ per cent.

These simple quantitative laws are readily applicable to the determination of molecular conditions in simple solutions. Most attempts to apply physical chemistry to pathology have been attempts to apply them, in a similar way, to complex biological solutions.

Probably the simplest illustration of such application is afforded by a study of ionisation. Thus, if NaCl is dissolved in water, its molecules, according to the newer chemistry, at once begin to break up, forming free atoms of Na and Cl. The rate of formation of these ions is determined by a law similar to that for the decomposition of cane sugar, and is expressed mathematically as follows:

$$[\text{Na}] = [\text{Cl}] = K \times [\text{NaCl}]\,,$$

where [Na] and [Cl] represent the number of free Na- and Cl-ions formed per unit of time, [NaCl] the number of unchanged NaCl molecules present in the solution at the beginning of that unit, and K a constant.

The ions so formed at once begin to recombine, forming anew molecular NaCl. The rate of recombination is determined by a law similar to that governing the reaction of methyl acetate and potassium hydrate, and is expressed mathematically as follows:

$$[\text{NaCl}] = K' \times [\text{Na}] \times [\text{Cl}]\,,$$

where [NaCl] represents the number of molecules of NaCl re-formed in unit time, [Na] and [Cl] the number of uncombined Na- and Cl-ions present in the solution at the beginning of that unit, and K′ a constant.

At first the amount of NaCl that decomposes per unit time is in excess of the amount re-formed in the same time; but, as larger and larger numbers of Na- and Cl-ions come to exist in the solution, the two rates become more nearly equal. Eventually a stage is reached in which new molecules are being formed as fast as old ones are being decomposed, that is, in which

$$\mathrm{K}' \times [\mathrm{Na}] \times [\mathrm{Cl}] = \mathrm{K} \times [\mathrm{NaCl}] .$$

This may be transposed to read

$$\frac{[\mathrm{Na}] \times [\mathrm{Cl}]}{[\mathrm{NaCl}]} = \frac{\mathrm{K}}{\mathrm{K}'} = K .$$

This equation expresses the numerical relation between the number of free and combined Na- and Cl-atoms in a simple NaCl-solution. It is one of the simplest formulæ whose application has been attempted in serum pathology. It has been conceived, for example, that the hemolytic substance ("hemolysin") present in hemolytic serum is a compound substance, formed by a union between the thermostable substance (amboceptor) and the thermolabile substance (complement) of such serum. It has been conceived that the union between these two components is similar to that between the Na- and Cl-atoms above, a state of equilibrium existing in which

$$\frac{[\text{Free amboceptor}] \times [\text{Free complement}]}{[\text{Combined amboceptor-complement}]} = K .$$

As an example of a more complex formula governing molecular distribution there may be cited the law governing the interaction of acids and bases. Here the acids and bases tend to form salts; but the salts so formed in turn tend to decompose, re-forming directly or indirectly the original acids and bases. When strong acids and strong bases are used, the tendency to decompose is practically negligible, when compared with the vigorous tendency to unite. With weak acids and weak bases, however, the tendency to decompose plays a relatively important rôle. Here it has been shown that, when certain weak acids and weak bases are put together, equilibrium is established in accordance with the formula

$$\frac{[\text{Free acid}] \times [\text{Free base}]}{[\text{Combined acid-base}]^2} = K .$$

This formula also has been applied in serum pathology. It has been conceived, for instance, that the interaction between toxins and antitoxins is similar to that between weak acids and weak bases, and that in mixtures of the two, equilibrium is established in accordance with the formula

$$\frac{[\text{Free Toxin}]\times[\text{Free antitoxin}]}{[\text{Combined toxin-antitoxin}]^2}=K\ .$$

Before attempting to examine the experimental evidence cited in support of these and similar formulæ, it will be necessary to point out certain fundamental properties of simple solutions to which the formulæ are demonstratedly applicable. In order to get convenient numbers for calculation, let us suppose that 702 grams of NaCl (=12 gram-molecules) are dissolved in a liter of water. How much of the NaCl will exist in the molecular form? How much as ionic Na and Cl?

As developed above, equilibrium will be established in accordance with the formula

$$\frac{[\text{Na}]\times[\text{Cl}]}{[\text{NaCl}]}=K\ .$$

It is found experimentally that for NaCl, at ordinary temperatures, $K=1$, provided [Na] and [Cl] are expressed as the number of gram-ions per liter, and [NaCl] as the number of gram-molecules per liter. If therefore x gram-molecules of NaCl are conceived to be ionised, there will be formed x gram-ions of Na and x gram-ions of Cl, and will be left in the solution $(12-x)$ gram-molecules of un-ionised NaCl. Substituting these values in the above formula, we obtain

$$\frac{x\times x}{12-x}=1\ ,$$

from which

$$x^2+x=12\ ,\quad \text{or } x=3\ .$$

When equilibrium is established, we therefore have three gram-molecules of NaCl ionised and $12-3$, or nine, gram-molecules in the molecular form. This gives rise to the verification formula

$$\frac{3\times 3}{9}=1\ .$$

Let us now suppose that two gram-ions of Cl are removed from the solution, as can readily be done by precipitation methods. What change will this bring about in the ionisation?

It is evident that equilibrium will no longer exist, since

$$\frac{3\times(3-2)}{9}=\frac{3\times1}{9}\text{ is not}=1\ .$$

To restore equilibrium further ionisation must take place, so as to increase the factors in the numerator and decrease the denominator. If y gram-molecules of NaCl are broken up in this secondary ionisation, there would then exist in the solution $(3+y)$ gram-ions of Na, $(1+y)$ gram-ions of Cl, and there would be left $(9-y)$ gram molecules of NaCl. The equilibrium formula would then be

$$\frac{(3+y)\times(1+y)}{(9-y)}=1\ ,$$

from which

$$y^2+5y=6\ ,\quad\text{or } y=1\ .$$

This gives rise to the verification formula

$$\frac{(3+1)\times(1+1)}{9-1}=\frac{4\times2}{8}=1\ .$$

To restore equilibrium, therefore, one additional gram-molecule of NaCl has to be ionised.

If now more ionic Cl is removed from the solution, further ionisation will necessarily take place. This will give rise to such formulæ as

$$\frac{6\times1}{6}=1\ ,\quad\frac{8\times\frac{1}{2}}{4}=1\ ,\quad\frac{9\times\frac{1}{3}}{3}=1\ ,\quad\frac{10\times\frac{1}{5}}{2}=1\ ,\quad\frac{11\times\frac{1}{11}}{1}=1\ ,\quad\text{etc.}$$

In this way it is possible to remove practically all the free Cl from the solution and have equilibrium constantly preserved by changes in ionic distribution.

A second fundamental fact, an understanding of which is necessary for our present purpose, is the fact that widely different solutions may contain the same amount of a certain molecular or ionic component. The following formulæ represent solutions of NaCl, each containing four gram-ions of Cl, but in no two of which the other components are equal:

$$\frac{\frac{1}{4}\times4}{1}=1\ ,\quad\frac{1\times4}{4}=1\ ,\quad\frac{4\times4}{16}=1\ ,\quad\frac{10\times4}{40}=1\ .$$

If from each of these solutions the four gram-ions of Cl are removed, readjustment will take place as above. In so far, however, as the amount of molecular NaCl differs in the four solutions, the amount of ionic Cl available for this readjustment, is not the same in the four solutions. From the first solution practically five gram-ions of Cl could be obtained by successive withdrawals; from the second, eight gram-ions; from the third, 20; and so on.

Although the amount of free Cl is the same in the four solutions, the amount of ionic Cl available for chemical action, differs widely. This includes not only the free Cl already present, but the potential Cl that may be formed from further dissociation of the molecular NaCl.

In a similar way solutions may contain the same amount of molecular NaCl, but widely different amounts of other elements. This is illustrated by the formulæ

$$\frac{\frac{1}{2}\times 24}{12}=1\,,\quad \frac{1\times 12}{12}=1\,,\quad \frac{2\times 6}{12}=1\,,\quad \frac{3\times 4}{12}=1\,,\quad \text{etc.}$$

If part or all of the molecular NaCl were removed from these solutions, equilibrium would be restored by a recombination of free Na- and Cl-ions. In this way, by successive withdrawals of NaCl-molecules, there could be obtained from the first solution practically 12½ gram-molecules of NaCl; from the second, 13 gram-molecules; from the third, 14; and so on.

The amount of molecular NaCl available for chemical purposes would include, not only the molecular NaCl already present, but the potential NaCl that could be formed from the recombination of the free Na- and Cl-ions present.

The error current in practically all attempts to apply formulæ of mass-action to serum pathology is the error of neglecting to take into account this readjustment of components as soon as equilibrium is disturbed. The toxic action of a toxin-antitoxin mixture would depend, if the physico-chemical concepts apply to such mixtures, not so much on the amount of free toxin present in the solution, as on the amount of available toxin in the mixture. The available toxin includes not only the free toxin, but the toxin that could be formed by a dissociation of the thus-far purely hypothetical toxin-antitoxin compound.

The toxic action of such a mixture can be taken as a measure of the amount of free toxin present in it only under one of two assumptions. First, that the toxin produces its effects without itself being removed from the solution or in any way altered while in the solution, so that equilibrium is in no ways disturbed. This is an unthinkable condition, as a chemical union between cellular substance and toxin is definitely proved. Or, second, it may be taken as a measure on the assumption that the rate of dissociation of the toxin-antitoxin compound is so slow as to give a negligible amount of free toxin during the time occupied by the experiment. This is an improbable assumption, as the guinea-pig experiments usually extend over four or five days.

Similarly, according to the physico-chemical concept, several sera may contain the same amount of "hemolysin,"* but with widely different amounts of free amboceptor and free complement. Here the hemolytic action would depend not only on the amount of "hemoslysin" already formed in the sera, but also on the amount of potential "hemolysin" that may be formed from the free amboceptor and free complement present, as soon as equilibrium is disturbed.

The hemolytic power of such a serum can be taken as a measure of the amount of formed "hemolysin" present in it only under one of two assumptions. First, that the "hemolysin" produces its effect without itself entering into chemical union with corpuscles, or in any way being changed, either chemically or in amount, so that the existing equilibrium is in no way disturbed. To say the least, this is an unproved assumption, and almost an unthinkable one. Second, the hemolytic power may be taken as a measure on the assumption that the rate of union of free amboceptor and free complement is so slow as to give a negligible amount of "hemolysin" during the time occupied by the experiment. This assumption is disproved by the fact that artificial amboceptor-complement mixtures have hemolytic powers as soon as made, the hypothetical union taking place with great promptness.

The experimental foundation for the application of physical chemistry to serum pathology rests on the hypothesis that the physiological, chemical, or toxic effect of an unknown solution is a measure of the amount of a single component present in that solution. If the laws of physical chemistry do govern serum phenomena, this hypothesis is necessarily untrue, as it fails to take into account the readjustment of components as soon as equilibrium is disturbed. Being untrue, there is no way at present discernible by which a single component in such solutions can be measured quantitatively. Consequently measurements thus far made must be regarded as only of historic interest to the physical chemist.

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*The term "hemolysin" is used in this paper to designate the hypothetical amboceptor-complement compound in hemolytic serum.

SUMMARY.

1. All attempts to apply physical chemistry to serum pathology have as their foundation the assumption that the physiological, chemical, or toxic effect of certain solutions is a direct measure of the amount of a certain free or bound component in those solutions.
2. This assumption neglects the readjustment of components that the physico-chemical laws themselves necessitate.
3. The assumption, moreover, rests on no experimental grounds, and experimental verification of it is practically out of the question, till toxins, antitoxins, and the like are isolated in a state of purity.
4. Measurements thus far made in serum pathology are therefore only of historic interest to the physical chemist.